abcam

ab217267

# Anti-Smad4 antibody [SP306] - C-terminal

Recombinant | RabMAb | Advanced Validation | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | SP306 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.503 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/100 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/50 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/50 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/450 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/450 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/450 |
| **Notes** | - |

## ChIC/CUT&RUN-seq

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 5 µg |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## Target data

See full target information SMAD4

**Function**

In muscle physiology, plays a central role in the balance between atrophy and hypertrophy. When recruited by MSTN, promotes atrophy response via phosphorylated SMAD2/4. MSTN decrease causes SMAD4 release and subsequent recruitment by the BMP pathway to promote hypertrophy via phosphorylated SMAD1/5/8. Acts synergistically with SMAD1 and YY1 in bone morphogenetic protein (BMP)-mediated cardiac-specific gene expression. Binds to SMAD binding elements (SBEs) (5'-GTCT/AGAC-3') within BMP response element (BMPRE) of cardiac activating regions (By similarity). Common SMAD (co-SMAD) is the coactivator and mediator of signal transduction by TGF-beta (transforming growth factor). Component of the heterotrimeric SMAD2/SMAD3-SMAD4 complex that forms in the nucleus and is required for the TGF-mediated signaling (PubMed:25514493). Promotes binding of the SMAD2/SMAD4/FAST-1 complex to DNA and provides an activation function required for SMAD1 or SMAD2 to stimulate transcription. Component of the multimeric SMAD3/SMAD4/JUN/FOS complex which forms at the AP1 promoter site; required for synergistic transcriptional activity in response to TGF-beta. May act as a tumor suppressor. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |

| | |
|---|---|
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**Patented technology**
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | Smad4 also known as DPC4 or MADH4 is a central protein in the TGF-beta signaling pathway with a molecular mass of approximately 60 kDa. It acts as a signal transducer that facilitates communication from the cell surface to the nucleus. Smad4 is broadly expressed in various tissues playing an important role in the regulation of cellular processes. It forms a complex with receptor-regulated Smads (R-Smads) to translocate to the nucleus where it influences gene transcription. |
| **Biological function summary** | Smad4 influences cell proliferation differentiation and apoptosis by mediating signals from TGF-beta cytokines. It is part of the Smad protein family acting as a transcriptional controller. Upon TGF-beta receptor activation Smad4 forms complexes with Smad2 and Smad3 translocating to the nucleus to regulate genes imperative for cellular homeostasis. Its role in cell cycle regulation underlines its contribution to normal cellular functions and its potential involvement in disorders. |
| **Pathways** | Smad4 operates within the TGF-beta pathway linking extracellular signals to nuclear transcription alterations. It participates in the regulation of epithelial-mesenchymal transition (EMT) a process important for development and tumor progression. In these pathways Smad4 interacts closely with Smad2 and Smad3 |

| | |
|---|---|
| | orchestrating various cellular responses to external stimuli through transcriptional management. |
| **Associated diseases and disorders** | Smad4 is highly related to cancer and juvenile polyposis syndrome. Mutations or deletions in Smad4 disrupt its function contributing to the progression of pancreatic cancer and colorectal cancer among others. Within these contexts Smad4 connects strongly to other proteins like p21 and cyclin-dependent kinase inhibitors which are important in cell cycle arrest and impede tumor growth. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

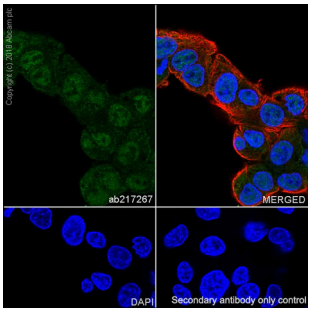
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

## 18 product images

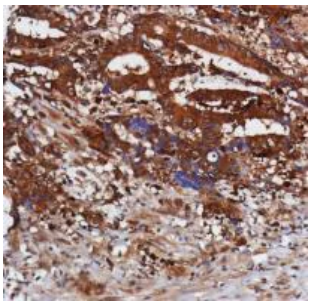



### Immunocytochemistry/ Immunofluorescence - Anti-Smad4 antibody [SP306] - C-terminal (ab217267)

Smad4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Smad4 antibody

Immunocytochemistry/ Immunofluorescence analysis of HepG2 (human hepatocellular carcinoma epithelial cell) cells labeling Smad4 with purified ab217267 at 1:50 (9 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at

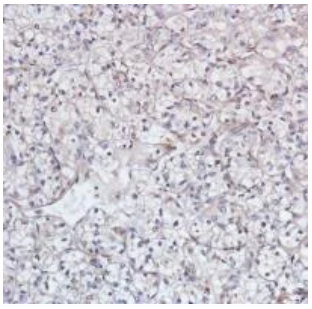
1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [SP306] - C-terminal (ab217267)

Smad4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad4 antibody

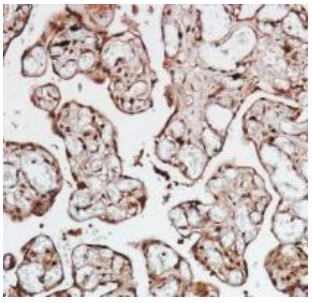
Immunohistochemical analysis of formalin-fixed, paraffin-embedded human colon adenocarcinoma tissue labeling Smad4 with ab217267 at 1/100 dilution.



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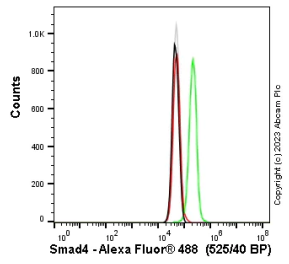
Immunohistochemical analysis of formalin-fixed, paraffin-embedded human renal cell carcinoma tissue labeling Smad4 with ab217267 at 1/100 dilution.



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [SP306] - C-terminal (ab217267)

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Immunohistochemical analysis of formalin-fixed, paraffin-embedded human placenta tissue labeling Smad4 with ab217267 at 1/100 dilution.




## Flow Cytometry (Intracellular) - Anti-Smad4 antibody [SP306] - C-terminal (ab217267)

Smad4 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad4 antibody

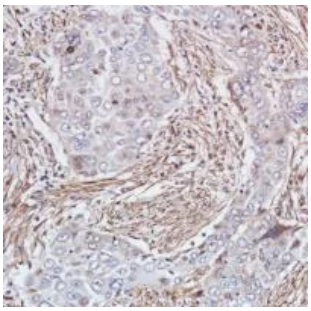
Flow cytometry overlay histogram showing wild-type Hap1 (green line) and SMAD4 knockout Hap1 stained with ab217267 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab217267) (1x 106 in 100µl at 0.008 µg/ml (1/265000)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, SMAD4 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

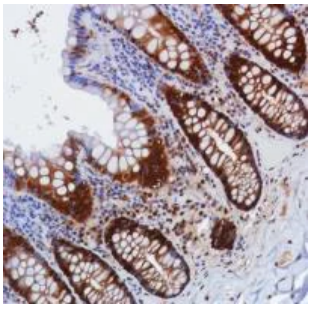
This antibody gave a positive signal in Hap1 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Smad4 antibody [SP306] - C-terminal (ab217267)

Smad4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Smad4 antibody

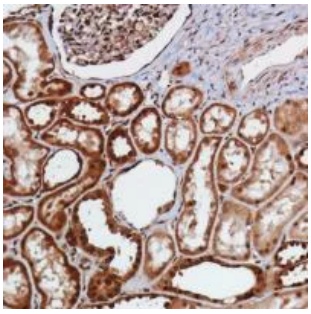
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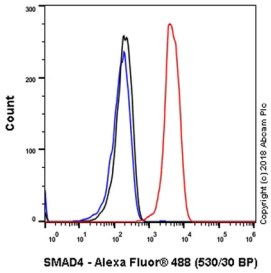
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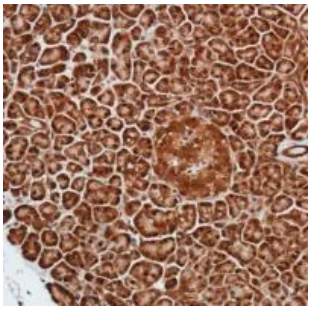
Immunohistochemical analysis of formalin-fixed, paraffin-embedded human kidney tissue labeling Smad4 with ab217267 at 1/100 dilution.




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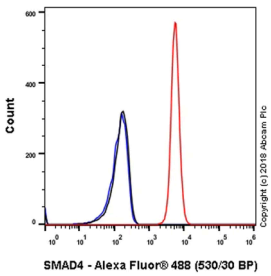
Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Smad4 with purified ab217267 at 1/450 dilution (1.01 µg/ml) Red. Cells were fixed with 4% paraformaldehyde . A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (ab172730) / Black. Unlabeled control - Unlabelled cells / Blue.



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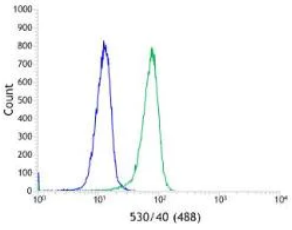
Immunohistochemical analysis of formalin-fixed, paraffin-embedded human pancreas tissue labeling Smad4 with ab217267 at 1/100 dilution.




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Smad4 Flow Cytometry (Intracellular) staining using rabbit Anti-Smad4 antibody

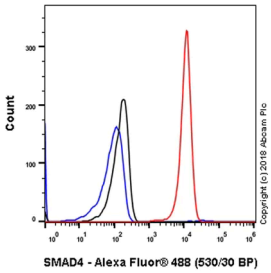
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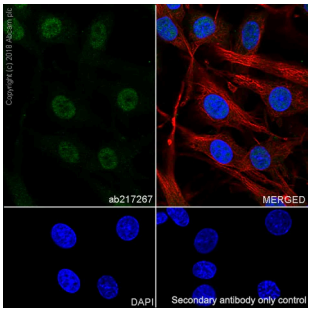
Intracellular flow cytometric analysis of NIH/3T3 (Mouse embryo fibroblast cell line) cellslabeling Smad4 with ab217267 at 1/400 dilution for 30 minutes at 4° C (green), compared to a negative control cell of rabbit IgG (blue).




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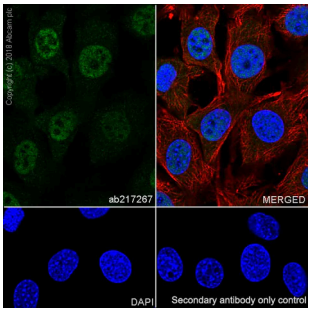
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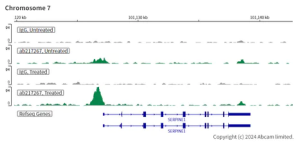
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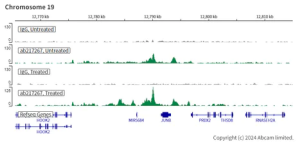
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## ChIC/CUT&RUN sequencing - Anti-Smad4 antibody [SP306] - C-terminal (ab217267)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x $10^5$ HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with hTGF-β1 (10 ng/mL 1 h) and 5 µg of ab217267 [SP306]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown.

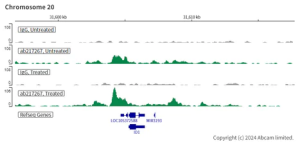
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



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